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Molecular genetic, life-history and morphological variation in a coastal warm-temperate sciaenid fish: evidence for an upwelling-driven speciation event

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ABSTRACT

Aim The Benguela Upwelling System (BUS) is a major biogeographical boundary in the south-eastern Atlantic, but little is known of the effect of historical permeability of the barrier on species distributed across the region. We used phylogenetic, life-history and morphological analyses to test the influence of regional oceanographic features on genetic divergence, evolutionary history and ecological divergence in a warm-temperate fish, *Atractoscion aequidens*.

Location Benguela Current, south-eastern Atlantic Ocean.

Methods Individual *At. aequidens* were analysed for molecular ($N = 35$), biological ($N = 81$) and morphological ($N = 282$) differentiation across the BUS region. To compare patterns of genetic divergence, we also included analyses of individuals sampled from three related *Argyrosomus* species occurring in the area.

Results *Atractoscion aequidens* comprises two deeply divergent lineages (average mtDNA genetic distance $p = 0.049$), which are separated by the boundary between the northern and southern Benguela subsystems. Divergence time was estimated at 2–3 Myr. Life-history traits, such as maximum size, growth rate and size and age at maturity, differed significantly between the two lineages, but morphological features did not. Divergent life-history traits appear to be associated with the evolution of migratory behaviour in the southern population, and thus ecological diversification across the Benguela region.

Main conclusions The oceanographic features of the BUS constitute an impermeable barrier to the dispersal of *At. aequidens* across the region. The estimated time since most recent common ancestor (TMRCA) points to the strengthening of the upwelling system in the last 2 Myr as the most likely mechanism driving isolation. However, the BUS is known to have periodic disruptions, which have mediated dispersal in multiple taxa. Therefore, the depth of genetic divergence and the presence of different life-history traits between the two lineages suggest that ecological divergence may have played a role in maintaining the observed isolation. Based on genealogical, geographical and ecological concordance, we suggest that *At. aequidens* is composed of two sibling species in the Benguela region and a full taxonomic revision of the genus is required.

Keywords

allopatry, *Atractoscion aequidens*, Benguela Current, biogeographical barrier, ecological divergence, marine biogeography

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INTRODUCTION

The marine environment is punctuated by biogeographical barriers that limit dispersal and gene flow in otherwise widespread species (Teske *et al.*, 2011a,b; Briggs & Bowen, 2012; Luiz *et al.*, 2012). These barriers may be physical obstacles such as landmasses (e.g. Isthmus of Panama) or less intuitive features such as deep water (Lessios *et al.*, 2003), freshwater outflows (Floeter *et al.*, 2008) or oceanographic features (Shaw *et al.*, 2004; Galarza *et al.*, 2009; von der Heyden *et al.*, 2011). Upwelling cells and sea surface temperature (SSTs) gradients in particular are known to disrupt gene flow, leading to divergence of allopatric populations and species (Waters & Roy, 2004; Teske *et al.*, 2011a; Henriques *et al.*, 2012, 2014, 2015). However, as oceanographic features are seldom permanent and frequently subject to considerable environmental variability, many barriers often permit some level of permeability to dispersal (Floeter *et al.*, 2008). Other processes may influence the persistence of differentiated allopatric taxa across such physical barriers (Bradbury *et al.*, 2008), with ecological divergence (and diversifying selection) being reported as a major evolutionary process influencing the biogeographical distributions of marine species (Pelc *et al.*, 2009; Teske *et al.*, 2011a; Gaither *et al.*, 2015).

In the south-eastern Atlantic, the Benguela Upwelling System (BUS) limits dispersion of marine fauna around southern Africa, and is recognized as an important biogeographical boundary between tropical taxa of the Indian and Atlantic Oceans (Floeter *et al.*, 2008). The present day features of BUS became fully established approximately 2 Ma (Shannon, 1985; Diester-Haass *et al.*, 1988), being characterized by reduced SSTs and a perennial upwelling cell off Lüderitz in central Namibia (Shannon, 1985) (Fig. 1). The upwelling cell forms the boundary between the northern and southern Benguela subsystems (Hutchings *et al.*, 2009), which in turn are bounded to the north and south by warm-temperate biogeographical zones created by the warm water Angola and Agulhas Currents respectively (Shannon, 1985).

Despite the complexity and obvious biogeographical importance of the BUS to the dispersal of tropical marine taxa, little is known regarding the permeability of this cold-water barrier to warm-temperate species. Disruption of the upwelling cell during periods of climatic change has been documented (e.g. Peeters *et al.*, 2004), implying that the BUS may constitute an incomplete barrier to dispersal, a premise supported by observation of different levels of apparent gene flow within different species across the region. For example, oceanic pelagic fishes do not exhibit significant genetic structuring across the region (Grant & Leslie, 2001; Teske *et al.*, 2011b), whereas coastal and migratory warm-temperate fishes exhibit differentiated allopatric populations to either side of the upwelling region (Emanuel *et al.*, 1992; Henriques *et al.*, 2012, 2014, 2015). In addition, the evolutionary and ecological consequences to fishes of the formation of this oceanographic system remain unquantified as little comparative molecular, morphological or life-history work has been

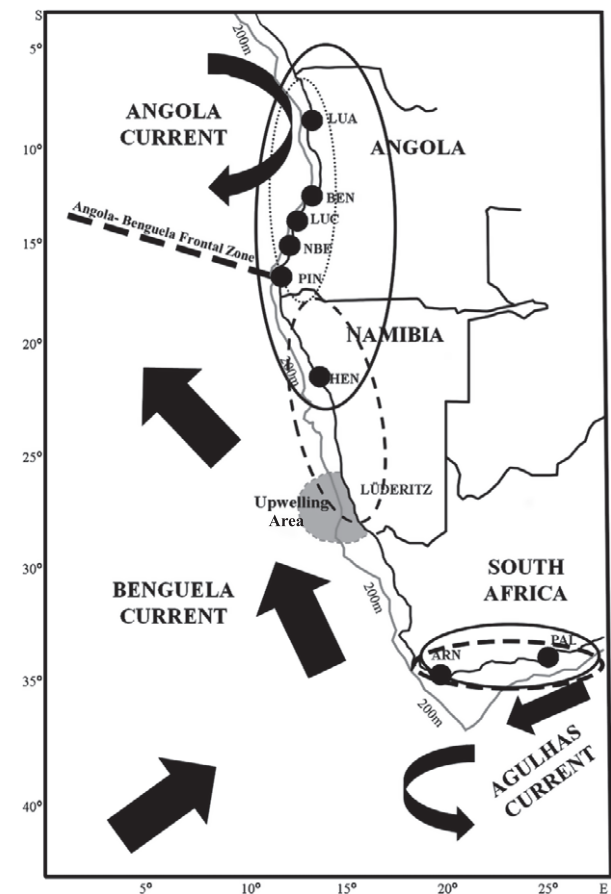


Figure 1 Sampling sites for *Atractoscion aequidens* around southern Africa, major oceanographic features of the Benguela Cold Current system, and sampled sciaenid species distribution ranges: *At. aequidens* = solid ellipses; *Argyrosomus coronus* = dotted ellipse (Angola); *Ar. inodorus* = dashed ellipse (Namibia); *Ar. japonicus* = rectangular dotted ellipse (South Africa). Sampling site codes: LUA, Luanda; BEN, Benguela; LUC, Lucira; NBE, Namibe; PIN, Pinda (Angola); HEN, Hentiesbaai (Namibia); ARN, Arniston; PAL, Port Alfred (South Africa).

conducted on warm-temperate species occurring across the region.

Geelbeck, *Atractoscion aequidens* (Cuvier, 1830), is a vagile, coastal sciaenid fish with a warm-temperate distribution, occurring around eastern Australia and south-western Africa (Sasaki, 1989). In southwest Africa, the species exhibits a disjunct distribution, with two documented centres of abundance confined to the northern and southern Benguela regions (Griffiths & Hecht, 1995) (Fig. 1). Absence of *At. aequidens* from southern Namibia and western South Africa is thought to be related to the presence of low SSTs, anoxic waters and a narrow continental shelf (Griffiths & Hecht, 1995). In the southern subsystem, the species undertakes seasonal reproductive and feeding migrations, which are likely linked to changes in availability of prey and seasonally changing SSTs (Griffiths & Hecht, 1995). Reproduction

appears to occur off the KwaZulu-Natal coast, from where long-lived pelagic egg and larval stages (135 days – Connel, 2007) are carried south in the Agulhas Current. In this region, the species grows to a large size (1300 cm total length) and matures at approximately 5 years of age (Griffiths & Hecht, 1995). No similar information is available for *At. aequidens* in the northern Benguela part of its range.

The biogeographical distribution implies that *At. aequidens* is composed of two allopatric populations situated within the warm-temperate boundaries of the northern and southern Benguela regions, and present day isolation and breakdown of gene flow between the two populations has been confirmed by Henriques *et al.* (2014). The potential temporal variability in the complex oceanographic features of the BUS and their effectiveness as a barrier to dispersal of marine species makes this an interesting system to: (1) examine the long-term permeability of such a barrier to gene flow and population connectivity in a migratory, warm-temperate coastal fish such as *At. aequidens*; and (2) investigate the likelihood of reproductive isolation and ecological/morphological divergence between allopatric populations to either side. We contextualize genetic divergence levels in *At. aequidens* by comparison with a related sciaenid species complex exhibiting similar life-history traits and biogeographical distribution (see Fig. 1): *Argyrosomus coronus* (Griffiths & Heemstra, 1995), *Ar. inodorus* (Griffiths & Heemstra, 1995) and *Ar. japonicus* (Temminck & Schlegel, 1843). In this study, we aimed to combine molecular genetic, biological and morphological techniques to investigate the patterns and processes of allopatric divergence across a potentially permeable marine biogeographical boundary.

METHODS

Sample collection

In total, 402 fish were collected from Angola, Namibia and South Africa (corresponding to the southern limit of the northern centre of abundance and the northern limit of the southern centre of abundance respectively) between 2008 and 2010 for phylogenetic, biological and morphological analyses (Fig. 1, see Table S1 in Appendix S1 in Supporting Information). To test extent and timing of population divergence, coalescent/statistical analyses (or reconstruction of phylogenetic relationships) were conducted for a subset of 35 individuals, from five locations in Angola, one in Namibia and two in South Africa (see Table S1 in Appendix S1). Three individuals each of *Ar. coronus*, *Ar. inodorus* and *Ar. japonicus* were collected from the same region. DNA was extracted using a phenol–chloroform method (Sambrook *et al.*, 1989) from fin clips preserved in 95% ethanol. For the biological survey, we collected monthly samples in southern Angola for comparison to the biological information for the southern population obtained from Griffiths & Hecht (1995). Morphometric analyses were performed for 85 fish from 10 localities in Angola and South Africa (see Table S1

in Appendix S1). Fish were photographed while fresh and fixed with 10% formaldehyde before transfer to 70% ethanol for storage and processing.

Phylogenetic analyses

DNA region amplification and sequencing

Extracted DNA was amplified by polymerase chain reaction (PCR) and sequenced for three mitochondrial DNA regions [control region (CR), cytochrome oxidase I (COI) and cytochrome b (cytb)], as different regions accumulate mutations at different rates (Avice, 2000), and one nuclear locus (first intron of the S7 ribosomal gene). Polymerase chain reactions were performed with universal primers for all loci using published protocols (CR: Apte & Gardner, 2002; COI: Ward *et al.*, 2005; cytb: Palumbi *et al.*, 2002; S7: Chow & Hazama, 1998). To eliminate amplification inconsistency, we developed species-specific primers for S7 (see Appendix S2). The PCR products were purified with an enzymatic digestion protocol (see Appendix S2), and sequenced with the same primers. Sequences were aligned and inspected in SEQUENCHER 4.7 (GeneCodes Corporation, Ann Arbor, Michigan, USA), and deposited in GenBank (*At. aequidens*: CR – JX192142-286; COI – JX1922549-586; Cytb – JX1922511-548; S7 – JX1922487-510; *Argyrosomus*: CR – JX191948/51/79/98, JX192004/16; COI – KC915055/45/44/77-79/66,65,71; cytb – KC915086,87,99/123-125/111,112,117; S7 – JX1922452-486). The DNA sequences of *Cynoscion nebulosus* (Sciaenidae) available on GenBank (CR: AF191147; COI: EU180146; cytb: GQ22013; S7: EU180162) were used as outgroup.

Alignment of *At. aequidens* and *Argyrosomus* sequences was unambiguous for all mtDNA loci. However, alignment of S7 sequences between genera proved difficult due to the presence of a variable length repeat region in *At. aequidens*. Therefore, *At. aequidens* and *Argyrosomus* spp. were analysed separately for the nuclear DNA (nDNA) dataset. Heterozygous positions (defined as two clear, equal sized peaks at the same position) in S7 sequences were observed at a frequency of 5.65% for the Angolan and Namibian samples, 0.33% for the South African samples and 1.00% for the *Argyrosomus* samples: haplotypes were statistically resolved using PHASE 2.1.1 (Stephens *et al.*, 2001), and only the most frequent (> 1%) were used.

Evolutionary history

Phylogenetic analyses of *At. aequidens* and *Argyrosomus* spp. datasets were conducted by concatenating all mtDNA fragments (mtDNA data), and for nDNA data (for each genus individually). We tested the alignments for heterogeneity of base composition using the χ^2 test in PAUP 4.0 (Swofford, 1993), and estimated the most likely model of nucleotide evolution in jMODELTEST 0.1 (Posada, 2008).

Reconstruction of phylogenetic trees used maximum likelihood (ML) and Bayesian approaches, performed in PHYL

3.0 (Guindon *et al.*, 2009) and MrBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001) respectively (see Appendix S2). A consensus tree was obtained after a burn-in of 2500 trees in TREEANOTATOR 1.6.1 (Drummond & Rambaut, 2007), and trees visualized in FIGTREE 1.3.1 (Rambaut, 2009). We reconstructed a median-joining network from the nDNA data using NETWORK 4.9.9.8 (Bandelt *et al.*, 1999).

As vicariant events can disrupt gene flow between adjacent populations and enhance genetic differentiation (Avise *et al.*, 1998), historical divergence between *At. aequidens* populations and among *Argyrosomus* species was estimated using uncorrected pairwise genetic distances in MEGA 4.1 (Kumar *et al.*, 2008).

Time since divergence

Time since most recent common ancestor for *At. aequidens* populations was estimated in BEAST 1.8.0 (Drummond & Rambaut, 2007) for the mtDNA COI region, and for the nDNA S7 region separately (see Appendix S2). Testing using MEGA 4.1 (Kumar *et al.*, 2008) indicated that all sequence data conformed to a model of constant evolutionary rate, so a strict molecular clock was enforced in all analyses. Due to the absence of a fossil record for *At. aequidens* and *Argyrosomus* spp. in the Benguela region, we used two approaches to calibrate a molecular clock (Drummond *et al.*, 2006): (1) applying fixed mutation rates for marine fishes, calibrated by the timing of the closure of the Isthmus of Panama, for both COI (1.2% Myr⁻¹ – Bermingham *et al.*, 1997) and S7 (0.46% Myr⁻¹ – Bernardi & Lape, 2005); and (2) employing the timing of intensification of the upwelling system in the BUS (c. 2 Ma) as an internal node calibrator for the divergence of *Ar. coronus* and *Ar. japonicus* (Henriques, 2012). The performance of each approach was assessed using posterior probabilities (Drummond & Rambaut, 2007), and convergence tested with effective sample size > 200. Mean divergence times and 95% credibility intervals (95% HPD) were estimated from the post burn-in results in TRACER 1.6 (Rambaut & Drummond, 2007).

Biological analyses

Fish were measured, sexed and staged macroscopically, and otoliths sectioned and aged, based on the criteria of Griffiths & Hecht (1995). As there were no raw data available for the South African study, we employed the age-length key of Griffiths (1988) to reconstruct the von Bertalanffy growth equation. To ensure comparability, we constructed an age-length key with identical length and mid-point classes for the Angolan samples. Modelling of growth followed Potts *et al.* (2008), and we used a likelihood ratio test (Cerrato, 1990) to compare the Angolan and South African fitted growth model parameters.

Estimates of size and age at maturity for the southern population were obtained from Griffiths & Hecht (1995), and we employed the standard logistic ogive method (Potts

et al., 2008) to estimate size at 50% and 100% maturity for the northern population. Age at maturity was estimated by converting length at maturity to age using the inverse von Bertalanffy growth equation. In addition, we calculated Roff's fecundity equivalent distance model ($FED = 125a^{0.2}GL^{(0.43 + 0.2b)}$), where a and b are values in the exponential length-weight equation, G is the maximum recorded gonadosomatic index and L is the length-at-50% maturity in cm) as a proxy for migration abilities for both *At. aequidens* populations (Roff, 1988).

Morphological analyses

Measurements and counts were based on Hubbs & Lagler (1947), with the amendments of Griffiths & Heemstra (1995), who conducted a morphological taxonomic evaluation of the genus *Argyrosomus* in the same region. We used six meristic character counts and 17 morphometric measurements. Meristic counts were tested using a Shapiro–Wilks Normality Test and means compared using a nonparametric t -test for independent groups. To remove the confounding factor of size (Humphries *et al.*, 1981), we excluded some larger South African individuals and used only fish with overlapping sizes (320–570 mm fork length) for the morphometric analysis. Although we recognize that the removal of large fish from the South African sample for the analysis may create bias in the results, if there are different allometric growth patterns between populations, this was considered necessary as the allometric effect associated with the inclusion of large South African specimens (not observed in Angola) would have led to a size bias in the morphometric comparison. Morphometric measurements were expressed as a ratio of standard (or head) length and were arc-sine transformed, then tested using Shapiro–Wilks normality tests with a significance level of $P = 0.05$. Principal component analyses (PCAs) were conducted in PAST 2.17 (Hammer *et al.*, 2001), using log-transformed morphometric measurements and meristic counts separately. Outliers were examined to see if they were representative of natural variation. To ensure that there was no bias due to maturity or sexual dimorphism, we first performed PCAs between juveniles and adults and then between sexes. As no significant results were found, we used the whole dataset to test for between-population variation.

RESULTS

Phylogenetic analyses

The three mtDNA fragments were concatenated into a single dataset of 1878 bp, with 348 variable sites and 71 bp identified as fixed differences between the two *At. aequidens* populations. Sequencing of S7 in *At. aequidens* resulted in a 279 bp fragment, with 23 variable sites and 8 fixed differences (after removing the length variable region). In *Argyrosomus* spp., S7 yielded a 463 bp fragment, with 73 variable and 6 fixed differences among species. Base frequency

homogeneity was not significant for either dataset ($\chi^2 = 17.263$, $P > 0.05$ for mtDNA; $\chi^2 = 10.652$, $P > 0.05$ for nDNA), indicating that sequences could be used for phylogenetic inference. Optimal models of nucleotide variation were: mtDNA – TN ($\alpha = 0.121$ – Tamura & Nei, 1993); *At. aequidens* nDNA – JC (Jukes & Cantor, 1969); *Argyrosomus* spp. nDNA – F81 (Felsenstein, 1981).

Maximum likelihood and Bayesian phylogenetic analyses of the mtDNA dataset produced highly resolved phylogenies (Fig. 2a). A reciprocally monophyletic division between two clades in *At. aequidens* representing Angolan-Namibian (northern) and South African (southern) populations, and reciprocally monophyletic divergence between all *Argyrosomus* spp., were retrieved with robust branch support (Fig. 2a), with no major (highly supported) divisions observed within clades or species. For the nDNA dataset, both the phylogenetic tree and the haplotype network revealed two divergent clusters within *At. aequidens*, displaying fixed differences and no shared haplotypes (Fig. 2b and Appendix S3). Although the nuclear dataset had to be analysed separately, branch lengths between the two *At. aequidens* clades and between *Ar. coronus* and *Ar. japonicus* were of a similar magnitude, suggesting a similar level of evolutionary divergence between the two *At. aequidens* lineages as between the two *Argyrosomus* species (see Appendix S3). On the contrary, *Ar. inodorus* appears to have diverged earlier (see Appendix S3).

Average uncorrected pairwise genetic distance (p) between northern and southern *At. aequidens* lineages was 0.049 for mtDNA and 0.042 for nDNA, lying within the range of mtDNA p values and substantially higher than the nDNA

p values observed among *Argyrosomus* species (Table 1 and Appendix S3). Mean sequence divergence between *At. aequidens* populations was approximately 10 times higher than divergence within populations (Table 2 and Appendix S3).

Time since divergence

Estimates of TMRCA between taxa based on the COI dataset were similar whether using an internal node calibration or the fixed mutation rate (Table 2). However, the 95% HPD interval was slightly broader for the former calibration. The TMRCA between the northern and southern *At. aequidens* clades was estimated at 1.83 Ma (1.22–2.49 Ma), which overlaps with the estimated TMRCA for the *Argyrosomus* spp. (Table 2). Enforcing an internal node calibration resulted in a mutation rate of 1.21% per Myr for *At. aequidens* COI, close to the fixed mutation rate for COI of marine fish species (Bermingham *et al.*, 1997).

Time since most recent common ancestor based on nDNA yielded an older divergence, which varied between 6.02 Ma

Table 1 Uncorrected pairwise genetic distances calculated from mtDNA and nDNA datasets between northern (AaA + AaN) and southern (AaSA) clades of *Atractoscion aequidens* and between *Argyrosomus* species (*Ar. coronus*, Ac; *Ar. inodorus*, Ai; *Ar. japonicus*, Aj), in the Benguela region.

	AaA-AaSA	Ac-Ai	Ac-Aj	Ai-Aj
mtDNA	0.049	0.054	0.041	0.065
nDNA	0.042	0.015	0.010	0.011

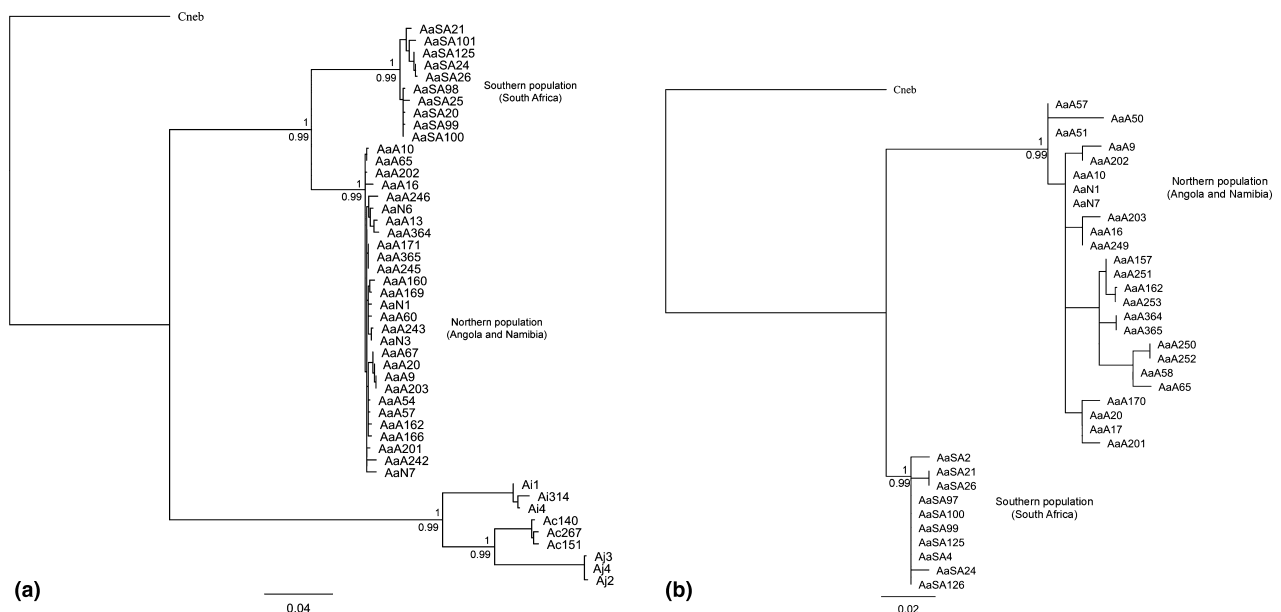


Figure 2 Reconstruction of phylogenetic relationships within *Atractoscion aequidens* and among *Argyrosomus* species from southern Africa, using concatenated mtDNA (a) and nDNA (for *At. aequidens* only) (b) data. Statistical support is indicated for nodes associated with major clades within *At. aequidens* or different species of *Argyrosomus* (Ai, Ac, Aj – see Table 1 for codes), given for both Bayesian (posterior probabilities above branches) and ML (aRLT – χ^2 values below branches) analyses. Outgroup: Cneb (*Cynoscion nebulosus*).

using the fixed mutation rate and 3.33 Ma using the internal node calibration (Table 3). In the latter, mutation rate increased to 0.55% per Myr, likely due to the presence of a variable size length region within the intron. Thus, the fixed mutation rate might not be applicable. The TMRCA based on nDNA for the *Argyrosomus* spp. retrieved the same divergence time as mtDNA (~2 Ma, Table 3), independently of the calibration method used.

Biological analyses

Mature fish were observed every month of the year in southern Angola, with the greatest proportion present between September and March. In contrast, peak reproductive season in the South African population was September–October (Table 4). Southern *At. aequidens* reached a larger maximum size but similar maximum age to that observed for northern individuals (Table 4). The results of the likelihood ratio test showed a significant difference in the rate (body growth coefficient) at which the northern and southern fish attained asymptotic length (L_{∞}). Significant differences between the populations were indicated for theoretical length at age zero (t_0 – $P = 0.04$), calculated asymptotic length (L_{∞} – $P < 0.01$) and the whole model ($P < 0.01$), suggesting that individuals in the southern population grow significantly faster than their northern counterparts (Table 4). Southern *At. aequidens* also matured at approximately double the length and double the age than compared with the northern fish (Table 4). Furthermore, Roff's FED model estimated migration at ~775 km for the southern population compared to ~50 km for the northern population.

Table 2 Uncorrected pairwise genetic distances, calculated from mtDNA and nDNA datasets, within northern (Aa) and southern (AaSA) clades of *Atractoscion aequidens* and within *Argyrosomus* species (*Ar. coronus*, Ac; *Ar. inodorus*, Ai; *Ar. japonicus*, Aj), in the Benguela region.

	AaA	AaSA	Ac	Ai	Aj
mtDNA	0.004	0.004	0.003	0.004	0.001
nDNA	0.012	0.002	0.002	0.001	0.003

Table 3 Estimates of time since divergence, calculated using two calibration methods (see text) from the COI data for the northern (AaA + AaN) and southern (AaSA) *Atractoscion aequidens* clades and for the assumed speciation events leading to the observed range of *Argyrosomus* species (Ac, Ai, Aj – see Table 1 for codes), in the Benguela region: Ln (likelihood), posterior likelihood of the calibration method employed; TMRCA, estimated time since most recent common ancestor in million years (95% HPD).

Marker	Calibration	Ln (likelihood)	TMRCA		
			AaA versus AaSA	Ai versus Ac/Aj	Ac versus Aj
mtDNA	1.2%	–5593.11	1.83 (1.22–2.49)	2.10 (1.44–2.82)	1.73 (0.90–2.74)
	2 Myr	–5717.56	1.88 (1.18–2.68)	2.136 (1.37–2.99)	1.72 (0.85–2.86)
nDNA	0.46%	–628.65	6.02 (3.05–9.54)	1.97 (0.99–3.11)	1.23 (0.32–2.03)
	2 Myr	–712.36	3.33 (2.08–4.68)	2.18 (0.89–3.68)	1.40 (0.53–2.38)

Morphological analyses

Meristic counts had similar ranges in both populations of *At. aequidens* for almost all characters (see Appendix S3). Even when the mode of the count differed (e.g. pectoral fin rays and lateral line scales), the ranges of counts for each population overlapped (see Appendix S3). The PCA extracted two components that explained 92.6% of variability in the meristic dataset, but when plotted showed no distinct separation between the two populations of *Atractoscion* (Fig. 3).

As with the meristic data, the ranges of body and head ratio measurements of fish belonging to the southern and northern populations overlapped. If size factor (first principal component) was removed, the fish did not cluster into distinct southern and northern populations (Fig. 4).

DISCUSSION

The Benguela Upwelling System and population isolation in a warm-temperate marine fish

The cold waters of the BUS have been perceived as a major biogeographical boundary isolating the tropical faunas of the Atlantic and Indian Oceans (Floeter *et al.*, 2008). The results of the present study, combined with previous population genetic studies across the same region (Henriques, 2012; Henriques *et al.*, 2012, 2014, 2015) suggest that the BUS may also constitute a barrier to dispersal and gene flow, resulting in population genetic and life-history character divergence, in warm-temperate coastal fishes.

Observed genetic divergence (p) between the lineages of *At. aequidens* inhabiting the northern and southern Benguela boundary regions was large for both mtDNA and nDNA datasets ($p = 0.049$ and $p = 0.042$ respectively). Similar levels of genetic divergence were observed here among distinct *Argyrosomus* species across the same area, and previously in recognized cases of speciation events across oceanographic barriers (Knowlton & Weigt, 1998; Donaldson & Wilson, 1999; Lessios *et al.*, 2003; Lessios, 2008; Grant *et al.*, 2010; von der Heyden *et al.*, 2011). Our results, therefore, suggest that the oceanographic features of the BUS represent a

Table 4 Comparative life-history characteristics of *Atractoscion aequidens* in South Africa (using reworked data from Griffiths, 1988) and Angola: Max. TL, maximum total length; Max. Wt, maximum mass; Max. age, maximum age; L_{∞} , asymptotic length; K , curvature parameter; t_0 , theoretical length at age zero; L_{50} , total length at 50%-maturity; A_{50} , age at 50%-maturity.

	Max. TL (mm)	Max. Wt (kg)	Max. age (years)	Spawning season	L_{∞}	K	t_0	L_{50} (mm)	A_{50} (years)
Angola	700	2.8	8	Sep–Mar	696	0.23	−2.57	452	2.2
South Africa	1300	25	9	Sep–Oct	1168	0.25	−1.60	953	5

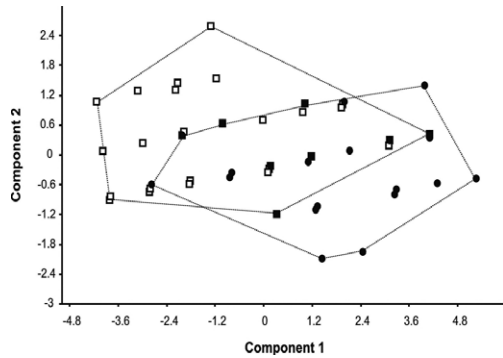


Figure 3 Scatterplot of the first and second principal component scores for the meristic characters of *Atractoscion aequidens* specimens from Angola (open squares) and South Africa (solid circles).

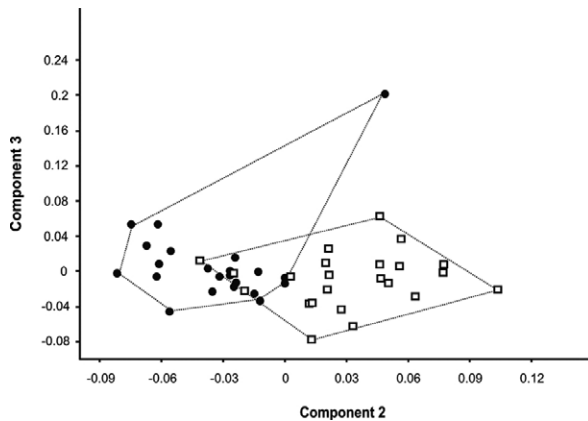


Figure 4 Second and third principal component scores for the morphometric measurements of *Atractoscion aequidens* from Angola (open squares) and South Africa (solid circles).

long-standing impermeable barrier to connectivity in *At. aequidens*.

Population isolation associated with oceanographic features

The distribution of the two allopatric *At. aequidens* populations around south-western Africa, and the genetic divergence between them, is associated with the geographical position of some, but not all, of the present day oceanographic features of the BUS. Adults of *At. aequidens* are

distributed across the boundaries between the Angolan and Agulhas warm currents and the cooler waters of the northern and southern Benguela subsystems (respectively), and the oceanographic fronts associated with these confluences, without evidence for breakdown of gene flow across these areas (Henriques *et al.*, 2014). However, the species is not found within the colder waters off southern Namibia, north-western South Africa and the perennial upwelling cell off central Namibia. These cold waters are predicted to be unsuitable habitat for adult *At. aequidens* as well as a barrier to dispersal of eggs and larvae (Lett *et al.*, 2007), and so we conclude they are the source of the isolation between the two populations.

The BUS developed 12–6 Ma (Diester-Haass *et al.*, 2002), with the present oceanographic features established by 2 Ma (Marlow *et al.*, 2000). Cyclical changes in sea level, water temperatures and upwelling intensity have occurred across the Benguela system during the past 6 Ma (Marlow *et al.*, 2000), changes that are often associated with population differentiation and speciation events (Avise *et al.*, 1998). Our estimated TMRCA between the *At. aequidens* clades varied substantially between mtDNA (1.8 Ma) and nDNA (6.02 Ma) sequences when applying widely used mutation rates for fish. In contrast, the TMRCA estimates for both mtDNA and nDNA sequences were similar for the *Argyrosomus* species (both indicating that isolation occurred around 2 Ma). This discrepancy between mtDNA- and nDNA-based estimates in *At. aequidens*, but not in *Argyrosomus*, is likely to result from different mutation dynamics in the two genera due to the presence of a hypervariable region in the S7 intron of *At. aequidens*, invalidating the use of the previously estimated mutation rate to calibrate a molecular clock for this DNA region in this species. Based on internal node calibration, TMRCA between the two *At. aequidens* lineages is more consistent between mtDNA and nDNA, and indicates isolation to have occurred between 3 Ma and 2 Ma during or soon after the establishment of the present day oceanographic features of the BUS at a time of intensification of the upwelling system (Marlow *et al.*, 2000), again pointing to the upwelling system as the source of isolation of *At. aequidens* subpopulations.

The BUS displays substantial temporal variation, usually associated with specific climatic patterns in the southern Atlantic (La Niña events), with years of decreased upwelling intensity and elevated SSTs (Kirst *et al.*, 1999). These events might be expected to promote dispersal across the upwelling area between the two Benguela subsystems, but an ancient

population divergence and the apparent absence of gene flow between northern and southern populations of *At. aequidens* are not consistent with permeability of this oceanographic barrier (Pelc *et al.*, 2009; Teske *et al.*, 2011a). Therefore, other evolutionary processes may have contributed to the genetic differentiation and ongoing isolation between *At. aequidens* populations (Bradbury *et al.*, 2008; Teske *et al.*, 2011a,b; Gaither *et al.*, 2015).

Allopatric population differentiation across the Benguela region: mechanisms and processes

Populations within species that are exposed to similar environmental conditions are likely to exhibit similar adaptive phenotypes and life-history features, but biological and ecological traits are expected to diverge in the case of isolation and exposure to new selective pressures, even in the presence of gene flow (Pelc *et al.*, 2009; Feder *et al.*, 2012; Martin *et al.*, 2013; Gaither *et al.*, 2015). Our biological analyses revealed that northern *At. aequidens* are smaller, grow more slowly and mature earlier than those in the southern Benguela subsystem. These alternative life-history strategies indicate the existence of two independently evolving lineages of *At. aequidens* in the BUS region. However, the reasons for diversification of these life-history traits are not clear, as other allopatric populations of fishes have not shown similar biological diversification across the same region (Potts *et al.*, 2008, 2010; Richardson *et al.*, 2011). Three mechanisms may explain the observed life-history divergence: different latitudinal distribution and associated SSTs; differing fishing pressures and ecological divergence.

Large and fast-growing fish species generally occur at higher latitudes, usually associated with colder SSTs (Macpherson & Duarte, 1994; Fisher *et al.*, 2010). However, as the northern and southern Benguela boundary regions exhibit similar SST ranges (<http://iridl.ldeo.columbia.edu/maproom/Global/Climatologies>), and other species across the region do not show similar life-history character divergence (Potts *et al.*, 2008, 2010; Richardson *et al.*, 2011), different latitude (i.e. colder SST in the southern area) is unlikely to be a contributing factor.

Commercial exploitation can also influence life-history traits (Heino & Godo, 2002; Richardson *et al.*, 2011). The southern population sustains extensive fishing pressures and is categorized as a collapsed stock (Winkler *et al.*, 2012). Although there is no comparable information for the northern subsystem, it is highly unlikely that this population has been exploited more heavily than its southern counterpart, which would be required to drive downwards the maximum size and size-at-maturity. Therefore, an exploitation-based hypothesis is also excluded.

Ecological divergence is most likely to explain differences in *At. aequidens* life-history characters. Unlike other coastal species with allopatric populations in this area, *At. aequidens* across the Benguela region display contrasting migratory behaviour. Southern *At. aequidens* undertake extensive

annual migration from the Eastern Cape to spawning grounds in KwaZulu-Natal (Griffiths & Hecht, 1995; Connel, 2007), whereas consistent catches of adults throughout the year and a protracted spawning season (W.M. Potts, unpublished data) suggest that northern *At. aequidens* do not migrate. Migration is known to be an adaptive response to differential ecological pressures, with recent studies suggesting that evolution of migration (or residency) is linked to different environmental conditions (Cresswell *et al.*, 2011; Shaw & Couzin, 2013). The evolution of migration in marine fishes is known to result in changes to life-history parameters such as increased maximum size, increased size-at-maturity and faster growth rates (Roff, 1988), as observed here in the migratory southern *At. aequidens* lineage. In support of the suggested link between migratory lifestyle and life-history characters, Roff's (1988) FED model predicted that the southern population should migrate ~775 km compared to ~50 km for the northern population, distances which correlate well with field observations (W.M. Potts, unpublished data). Therefore, a combination of genetic isolation and ecological diversification, the latter possibly driven by the evolution of migratory behaviour in southern fish, may explain the differential growth rates, body sizes, and age and size at maturity of *At. aequidens* populations. A similar combination of allopatric divergence driven by oceanographic features and associated ecological adaptation has been documented for several other marine species around southern Africa (Teske *et al.*, 2011a,b; von der Heyden *et al.*, 2011), and across other biogeographical boundaries (Shaw *et al.*, 2004; Galarza *et al.*, 2009; Gaither *et al.*, 2015).

Cryptic speciation in the Benguela region

Contrary to the observed genetic and life-history differentiation, morphological analyses did not differentiate fish belonging to northern and southern *At. aequidens* clades. The same pattern has been found in other fish species (e.g. Colborn *et al.*, 2001), and particularly in sciaenids (Santos *et al.*, 2006), suggesting that morphological change and divergence occurs at a slower pace than molecular genetic or life-history evolution in some species (Colborn *et al.*, 2001). The extent of genetic divergence between the northern and southern lineages, comparable to that observed between recognized *Argyrosomus* species across the same area, in combination with distinct life-history traits suggests that *At. aequidens* may comprise two morphologically cryptic species in the Benguela region. Therefore, the present results suggest a taxonomic revision of the *Atractoscion* genus is needed.

CONCLUSIONS

Molecular genetic, life-history trait and morphological analyses of *At. aequidens* around south-western Africa indicate that the Benguela Upwelling System represents an impermeable barrier to population connectivity in this warm-temperate marine fish. The oceanographic features of the system,

particularly regions of cold SSTs associated with the perennial upwelling cell, are most likely the primary mechanism affecting the contemporary *At. aequidens* biogeographical distribution, but levels of genetic and life-history divergence suggest that ecological divergence also may have played a role in maintaining isolation. Genetic divergence between the northern and southern Benguela region populations is equivalent to that between other sciaenid species across the same region, which together with clear life-history differences between populations suggests that *At. aequidens* may be composed of two sibling species around southern Africa, and that a full taxonomic revision of the genus is necessary.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Additional sampling information.

Appendix S2 Additional methods.

Appendix S3 Additional results.

BIOSKETCH

Romina Henriques is interested in the influence of oceanographic systems, such as the Benguela Upwelling System, on population dynamics and evolutionary history of marine fishes. Her work employs population genetic and phylogenetic approaches to understand the mechanisms behind evolution in the marine environment. This work began as part of her doctoral dissertation at the Centre for Ecology, Evolution and Behaviour, at Royal Holloway University of London.

Author contributions: R.H., W.M.P., W.H.S., C.V.S. and P.W.S. designed the study. R.H., W.M.P. and J.K. conducted the fieldwork. R.H. designed and performed the molecular lab work and analyses, and J.A.T. performed the bayesian phylogenetic analyses. W.M.P. designed the biological and morphological work, which was performed by W.M.P., and J.K. R.H., W.M.P. and P.W.S. drafted the manuscript, which was approved by all authors.

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